

CLAIMS:

1. An isolated nucleic acid sequence encoding MART-1.
2. The nucleic acid sequence of claim 1 having the sequence shown in Figure 1 (SEQ ID NO: 1).
3. The nucleic acid sequence of claim 1 wherein said sequence is an allelic variation of the sequence shown in Figure 1 (SEQ ID NO: 1).
4. The nucleic acid sequence of claim 1 wherein said sequence is a homolog of the sequence shown in Figure 1 (SEQ ID NO: 1).
5. The nucleic acid sequence of claim 1 wherein said sequence is a variant of the sequence in Figure 1 (SEQ ID NO: 1).
6. A recombinant protein encoded by the nucleic acid sequence of claim 1.
7. A recombinant protein encoded by the nucleic acid sequence of claim 2.
8. A recombinant protein encoded by the nucleic acid sequence of claim 3.
9. A recombinant protein encoded by the nucleic acid sequence of claim 4.
- 30 10. A recombinant protein encoded by the nucleic acid sequence of claim 5.

11. An isolated and purified protein comprising the amino acid sequence shown in Figure 1 (SEQ ID NO: 2) or a substantially homologous sequence thereof. 2
- 5 12. A peptide having the sequence AAGIGILTV (SEQ ID NO: 4), EAAGIGILTV (SEQ ID NO: 17), or AAGIGILTVI (SEQ ID NO: 18). 3
- 10 13. A method of producing the recombinant protein, according to claim 1, comprising:
- 15 (a) inserting the nucleic acid sequence shown in Figure 1 (SEQ ID NO: 1) sequence into an expression vector;
- (b) transferring the expression vector into a host cell;
- (c) culturing the host organism under conditions appropriate for amplification of the vector and expression of the protein; and
- (d) harvesting the protein.
- 20 14. The method of claim 13, wherein the expression vector is a eukaryotic expression vector or prokaryotic expression vector.
- 25 15. The method of claim 13, wherein the expression vector is a baculovirus vector.
16. The method of claim 13, wherein the host cell is a eukaryotic cell or prokaryotic cell.
- 30 17. The method of claim 13, wherein the eukaryotic cell is an insect cell.
18. A recombinant expression vector comprising all or part of the nucleic acid sequence of claim 1.
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19. A host organism transformed or transfected with the recombinant expression vector according to claim 18 in a manner to allow expression of said protein encoded by said recombinant expression vector.
20. Antibodies reactive with the protein according to claim 11 or portions thereof.
21. The antibodies of claim 20 wherein said antibodies are monoclonal.
22. The antibodies of claim 20 wherein said antibodies are polyclonal.
23. A method for detecting MART-1 messenger RNA in a biological sample comprising the steps of:
  - (a) contacting all or part of the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) with said biological sample under conditions allowing a complex to form between said nucleic acid sequence and said messenger RNA
  - (b) detecting said complexes; and
  - (c) determining the level of said messenger RNA.
24. The method of claim 23 wherein said sample is selected from the group consisting of mammalian tissues, mammalian cells, necropsy samples, pathology samples and biopsy samples.
25. The method of claim 24 wherein said biological sample is from a mammal afflicted with a disease state.
26. The method of claim 25 wherein said determination of said level of said mRNA is used to diagnose, assess or prognose the disease state.

27. The method of claim 26 wherein said biological sample is from a mammal afflicted with melanoma or metastatic melanoma.
- 5 28. A method of detecting MART-1 protein in a biological sample comprising the steps of:  
(a) contacting a reagent which specifically reacts and forms a complex with said protein in said sample; and  
10 (b) detecting the formation of said complex between said protein and said reagent.
- 15 29. The method of claim 28 wherein said sample is selected from the group consisting of mammalian tissues, mammalian cells, necropsy samples, pathology samples, and biopsy samples.
- 20 30. The method of claim 28 wherein said reagent is an antibody or fragment thereof.
- 25 31. The method of claim 28 wherein said reagent is monoclonal antibody.
32. The method of claim 28 wherein said reagent is a polyclonal antibody.
- 25 33. The method of claim 28 wherein said biological sample is from a mammal afflicted with a disease state.
- 30 34. The method of claim 28 wherein said determination of said level of said protein is used to diagnose, assess or prognose the disease state.
- 35 35. The method of claim 34 wherein said disease state is melanoma or metastatic melanoma.

36. A method of detecting MART-1 genomic nucleic acid sequences in a biological sample comprising the steps of:
- (a) contacting all or part of the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) with a biological sample under condition to allow complexes to form between said nucleic acid sequence and said genomic DNA sequences; and
- (b) determining alterations in said genomic sequence.
37. The method of claim 36 wherein said alteration is a deletion, substitution, addition or amplification of said genomic DNA sequences.
38. An immunogenic peptide having contiguous amino acids derived from the MART-1 sequence (SEQ ID NO: 2).
39. The immunogenic peptides of claim 38 wherein such peptides are at least about 9 to 10 amino acids in length.
40. The immunogenic peptide of claim 39 where said peptide has the sequence AAGIGILTV (SEQ ID NO: 4), EAAGIGILTV (SEQ ID NO: 17) or AAGIGILTVI (SEQ ID NO: 18) or an analog thereof.
41. An immunogenic peptide having contiguous amino acids derived from the gp100 sequence (SEQ ID NO. 27).
42. The immunogenic peptide of claim 41 wherein said peptide is at least about 9 to 10 amino acids in length.
43. The immunogenic peptide of claim 42 having the sequence LLDGTATRL (<sup>(SEQ ID NO: 31)</sup> ~~(SEQ ID NO: 33)~~), VLYRYGSFSV (<sup>(SEQ</sup>

Seq ID No: 32

(Seq ID No: 34)

~~SEQ ID NO: 34), VLKRCLLHL (SEQ ID NO: 36), ALDGGGNKHFL  
(Seq ID No: 33), (Seq ID NO: 35), VLPSPACQLV (SEQ ID NO: 37), or  
(Seq ID No: 36), SLADTNSLAV (SEQ ID NO: 38).~~

- 5        44. The immunogenic peptide of claims 38 or 41 wherein  
said peptide is recognized by HLA-A2 restricted tumor  
infiltrating lymphocyte.
- 10      45. The immunogenic peptide of claims 38 or 41 wherein  
said peptide is a native, synthetic or recombinant  
peptide.
- 15      46. A pharmaceutical composition comprising the  
recombinant proteins of claim 6 and an acceptable  
excipient, diluent or carrier.
- 20      47. A method of preventing or treating melanoma  
comprising administering the pharmaceutical  
composition of claim 46 to a mammal in an effective  
amount to stimulate the production of protective  
antibodies or immune cells.
- 25      48. A vaccine for immunizing a mammal comprising a  
recombinant protein according to claim 6 in a  
pharmacologically acceptable carrier.
- 30      49. A pharmaceutical composition comprising the peptides  
of claims 38 or 41 and a suitable excipient, diluent  
or carrier.
- 35      50. A method of preventing or treating melanoma  
comprising administering the pharmaceutical  
composition of claim 49 to a mammal in an effective  
amount to stimulate the production of protective  
antibodies or immune cells.

51. A method of identifying genes encoding melanoma antigens using tumor infiltrating lymphocytes (TIL), said method comprising the following steps:
- (a) isolating tumor infiltrating lymphocytes from a tumor from a mammal afflicted with melanoma;
  - (b) introducing a melanoma cDNA library into a mammalian cell line;
  - (c) exposing said mammalian cells (from step 5) to said TIL;
  - (d) screening for expression of an antigen encoded by said cDNA in said mammalian cells recognized by said TIL; and
  - (e) isolating said cDNA corresponding to said antigen.
52. The method of claim 51 wherein said cells in step (b) are selected from the group consisting of tumor cell lines or COS 7 cells.
53. A method for assessing immunogenicity of peptides derived from amino acid sequences of a MART-1 protein having the sequence (Figure 1; SEQ ID NO: 2) or a gp100 protein having the sequence (Figure 5A; SEQ ID NO: 27) said method comprising the steps of:
- (a) preparing a plurality of peptides based on the MART-1 or gp100 amino acid sequence;
  - (b) incubating at least one of said peptides with a mammalian cell line;
  - (c) exposing said mammalian cells incubated with said peptide to tumor infiltrating lymphocytes (TIL); and
  - (d) screening for recognition of TIL with said cells incubated with said peptide.
54. The method of claims 53 wherein said peptides in step (a) are about 9 to 10 amino acids.

55. The method of claim 53 wherein said cells in step (b) are selected from the group of COS cells, T2 cells, or EBV transformed B cell lines.
- 5 56. A purified and isolated nucleic acid sequence encoding a peptide comprising at least about 8 contiguous amino acids, said peptide being derived from the MART-1 sequence (Figure 1; SEQ ID NO: 2) or the #gp100 sequence (Figure 5A; SEQ ID NO: 27), said peptide being reactive to tumor infiltrating lymphocytes (TIL).
- 10 57. A recombinant expression vector comprising? at least one nucleic acid sequence of claim 56.

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